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CENTROMERIC HETEROCHROMATIN IN *LACHENALIA TRICOLOR* (L.) THUNB.

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Abstract

The South African *Lachenalia tricolor* is notable for possessing large amounts of heterochromatin. The heterochromatin is centromeric in position, and may be revealed either by direct quinacrine staining or by denaturation-reannealing treatments. The significance of this is discussed relative to the general occurrence and adaptive significance of this type of chromatin.

Uittreksel

SENTROMERIESE HETEROCHROMATIEN IN *LACHENALIA TRICOLOR* (L.) THUNB.

Die Suid-Afrikaanse *Lachenalia tricolor* word deur groot hoeveelhede heterochromatien gekenmerk. Die heterochromatien is sentromeries in plasing en kan deur direkte beitsing met quinakrien of met denaturasie-hervoeging behandeling aangedui word. Die betekenis hiervan word bespreek met betrekking tot die algemene voorkoms en aanpasbaarheidsbetekenis van dié soort chromatien.

Introduction

Heterochromatin, i.e. the late-replicating fraction of DNA present as condensed "chromocentres" in resting nuclei, has been the subject of renewed interest over recent years. This has followed the discovery that the positions of heterochromatin bands in metaphase chromosomes may be revealed by their differential fluorescence in ultraviolet light following treatment with certain fluorochromes (Caspersson *et al.*, 1968), and by their differential staining with Giemsa or orcein following denaturation-reannealing treatments (Pardue and Gall, 1970; Vosa, 1973a). The latter method derives from the highly repetitive base constitution of heterochromatin, which results in its reannealing more completely than the remainder. These techniques are of much wider applicability than the older method based on the understaining of heterochromatin in chromosomes exposed to low temperature (Darlington and La Cour, 1940), for only certain types of heterochromatin show such cold sensitivity.

The new techniques have greatly increased the number of plant species in which heterochromatin is known to occur. Heterochromatin has been demonstrated, for example, in *Vicia* (Vosa and Marchi, 1972), *Hepatica, Anemone* (Marks and Schweizer, 1974), and *Encephalartos* (Mogford, 1978). Heterochro-

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matin has been found to be particularly frequent among the cultivated cereals such as *Triticum* (Gill and Kimber, 1974), *Secale* and *Hordeum* (Vosa, 1974, 1976a), and also among the Liliaceae such as in *Scilla, Tulbaghia* and *Allium* (Vosa, 1973 b,c, 1976 b,c).

In many such cases it has become clear that the number and position of heterochromatin bands constitutes a character as important in the study of species relationships as are chromosome number and morphology themselves. Equally, however, among plant species the occurrence of heterochromatin in large amounts remains exceptional. The discovery of large quantities of heterochromatin in the South African *Lachenalia tricolor*, to be described in the present work, was therefore considered of particular interest.

MATERIAL AND METHODS

Investigations were performed on plants from a clonal population of *Lachenalia tricolor* growing in the garden of the Department of Plant Sciences, Rhodes University, and on plants of the variety *quadricolor* obtained from a commercial source. Voucher specimens of these varieties are deposited in the Rhodes University Herbarium as accessions number *23793* and *23794* respectively.

In all cases rapidly growing root tips were used, pretreated with 0,1 % colchicine for four hours and fixed in 3:1 ethanol: glacial acetic acid for 8-16 hours.

For studies of chromosome number and morphology, root tips were hydrolysed in 1N HCl at 60 °C for 6 minutes, incubated in Feulgen reagent for 90 minutes at room temperature, and squashed in acetic orcein.

For the recognition of heterochromatin, three techniques were used: quinacrine fluorescence (Caspersson *et al.*, 1968); denaturation-reannealing followed by staining with the benzimidazole derivative Hoechst 33258 (Vosa, 1976b); and denaturation-reannealing followed by Giemsa staining (Pardue and Gall, 1970).

The procedures were as follows. For all methods except quinacrine staining, the root tips were first hydrolysed in 0,2N HCl at 60 °C for 2,5 minutes. For all treatments, the root tips were then squashed in 45 % acetic acid under an albumenised coverslip, the coverslip then being floated off in absolute alcohol and air dried. Subsequent steps were conducted on the cells adhering to the coverslip.

For quinacrine fluorescence, the preparations were stained in 0,5 % quinacrine dihydrochloride (Gurr's Atebrine) in absolute alcohol for 5 minutes at room temperature. Excess quinacrine was rinsed off in absolute alcohol, and the preparation air-dried, following which the preparation was mounted in 50 % glycerine and viewed using a Zeiss fluorescence microscope with exciter filter BG12 and barrier filters 50 plus 53.

For the denaturation-reannealing treatments, the preparations after the first air-drying were denatured by immersion for 6 minutes in a saturated solution of barium hydroxide at room temperature. The preparations were then rinsed thoroughly in running distilled water, and reannealed by immersion in 2 × saline sodium citrate buffer for 30 minutes at 60 °C. The preparations were then either stained in 0,02 % alcoholic Hoechst 33258, with this and the subsequent steps as for quinacrine staining, or else were immersed overnight in a 0,5 % aqueous solution of Giemsa buffered to pH 6,8. The latter preparations were then air dried and mounted permanently in Gurr's Depex.

RESULTS

The diploid chromosome number was found to be 14, as previously determined by Spelta (1972). All the chromosomes were very small, ranging in size from 1,1 to 2,6 μ. The karyotype was markedly asymmetric, being composed of one, relatively large, telocentric pair (pair 1, Fig. 1), one large and two small acrocentric pairs (nos. 3, 4 and 5), and one large and two small metacentric pairs (nos. 2, 6 and 7).

The techniques for heterochromatin recognition indicated that heterochromatin was present on all the chromosomes, and was exclusively centromeric in position. The heterochromatin was uniform in its staining response, showing positive stainability in all three techniques.

Of the various techniques, quinacrine staining was the least satisfactory, due to the rapid fading of fluorescence. In contrast, denaturation-reannealing followed by Hoechst staining gave a brilliant, stable fluorescence which clearly indicated the location of heterochromatin despite the small size of the chromosomes. The results with Giemsa were comparable but less distinct.

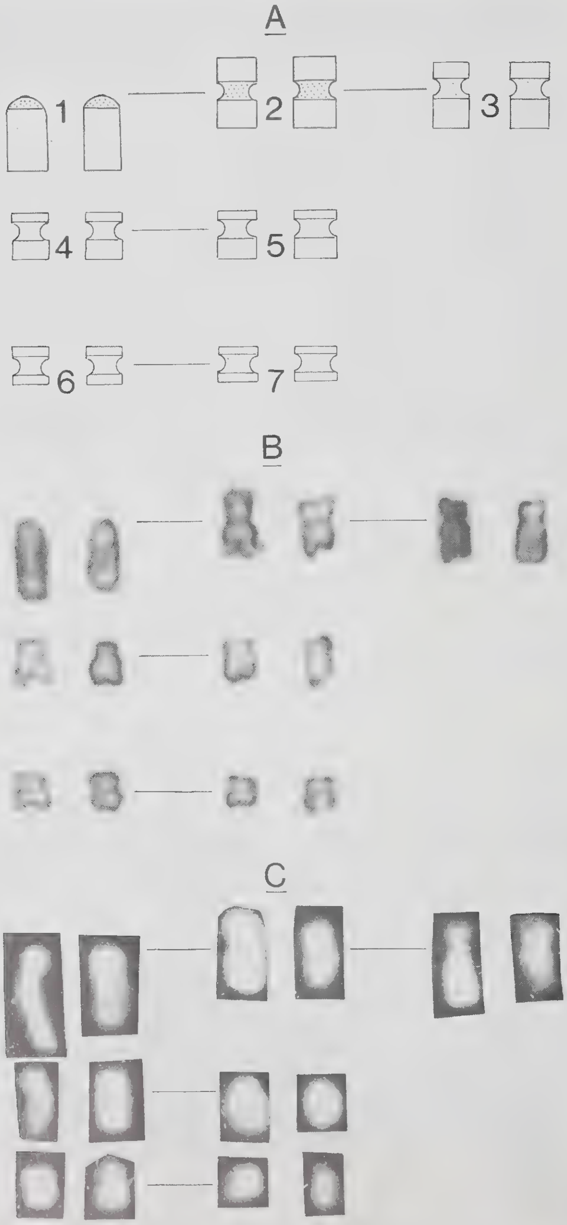
Chromosome number, morphology, and the position of heterochromatin were all constant throughout both cultivars studied.

DISCUSSION

The present work emphasises further the widespread occurrence of heterochromatin among the Liliaceae, the significance of which is not yet clear. Stebbins (1971) regarded heterochromatin as particularly characteristic of highly specialised species—a viewpoint which, though fully supported by the recent demonstration of heterochromatin in *Encephalartos* (Mogford, 1978), is nevertheless difficult to apply to individual species such as the present without adequate comparative studies within the groups concerned.

From the purely functional viewpoint, it is of interest that all the heterochromatin in the present species is centromeric in position. Such is the case even with the telocentric chromosomes, which morphologically are highly dissimilar to the remainder. Terminal and intercalary bands, so frequent in the other cases cited above, are not in evidence.

Moreover, no differences occurred between chromosomes in the staining responses of the heterochromatin. This contrasts with the situation which exists, for example, in *Allium flavum*, in which different heterochromatin bands show all


A
B
C

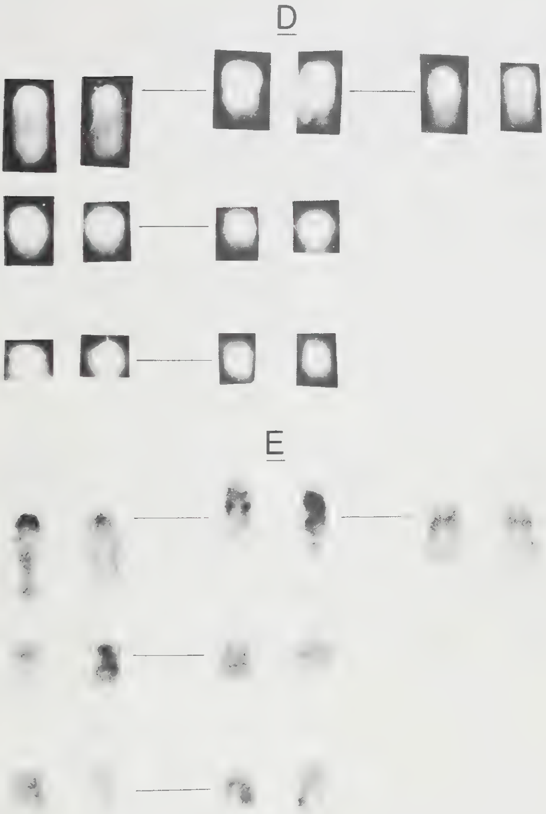


FIG. 1.
The karyotype of *Lachenalia tricolor*

A, Idiogram. Stippling indicates position of heterochromatin; B, Feulgen/Acetic orcein staining, phase contrast illumination; C, Quinacrine, fluorescence; D, Barium/SSC/Hoechst, fluorescence; E, Barium/SSC/Giemsa, brightfield illumination.
All × 1 500.

four combinations of enhanced or reduced quinacrine fluorescence, and positive or negative stainability with Giemsa (Vosa, 1973d).

The constant position and stainability of heterochromatin in this species suggests that such has a uniform and specific role. Brown (1966) suggested that heterochromatin in the vicinity of the centromeres, and also that in the vicinity of the nucleolar organisers, might serve to separate off the highly specialised activities of these regions from those of the remainder of the chromosome.

Nowadays, however, other possibilities may be cited. Heterochromatin, for instance, is now known to be characterised by a highly repetitive base constitution, which suggests that nucleolar organiser heterochromatin might be involved in nucleolar synthesis (Mogford, 1978); while centromeric heterochromatin might well be involved in chromosome organisation during interphase, or meiotic pairing (Mogford, 1977). Further work is necessary to examine these possibilities.

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